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| 学位の種類 | 博士（理学） |
| 学位記番号 | 理学博　第1号 |
| 学位授与の日付 | 平成31年2月14日 |
| 課程・論文の別 | 学位規則第4条第1項該当 |
| 学位論文題名 | Studies on the effects of unsaturated fatty acids on epidermal keratinocytes<br>表皮角化細胞における不飽和脂肪酸の影響に関する研究 |
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**【論文の内容の要旨】**

**Introduction**

Sebum is produced in sebaceous glands and secreted to the skin's surface through hair follicle ducts. An appropriate amount of sebum is thought to have a moisturizing effect on the skin. On the other hand, excessive sebum has a bad influence such as abnormal epidermal differentiation in acne vulgaris. Conspicuous facial pore are also skin complications that are thought to be associated with excessive sebum. Although excessive sebum is related to the abnormal differentiation, the mechanism involved has not been clarified.

Human sebum is composed of wax esters, squalene, triglycerides, and free fatty acids. Of these components, triglycerides and free fatty acids together amount to about 60% of total human sebum. The free fatty acids are released from triglycerides in the sebum by bacteria such as *Propionibacterium acnes*. Free fatty acids in sebum are composed of saturated and unsaturated fatty acids. C16 and C18 fatty acids are especially predominant. Some of the sebum components are presumed to be the cause of the abnormal differentiation.

In this study, the effects of sebum components on epidermal differentiation were investigated. First, the relationship between conspicuous facial pores and sebum composition was investigated. Next, in order to identify the components which induce the abnormal differentiation, the effects of the lipid components in sebum were

evaluated in vivo mouse skin and in vitro human cultured keratinocytes. Finally, the mechanisms of abnormal epidermal differentiation caused by the sebum components were investigated.

## Results

### 1. Relationship between conspicuous facial pores and the sebum components

First, a skin measurement test was conducted using 59 Japanese women volunteers in their 20s and 30s. The volunteers were divided in three groups according to the degree of conspicuity of the pores: (A) Inconspicuous group, (B) Normal group, (C) Conspicuous group. Sebum of the volunteers was collected and analyzed by gas chromatography. The total sebum amount and the proportion of unsaturated fatty acids were increased in the conspicuous group. The trans-epidermal water loss (TEWL) value and number of parakeratotic corneocytes were high in the conspicuous group, indicating that the epidermal differentiation is abnormal. This result suggests that sebum secreted from facial pore damages the stratum corneum around facial pore. Should this be the case, stratum corneum around facial pore might be worse than that of the inter-follicular areas. To address this issue, we next compared the stratum corneum around facial pores and the inter-follicular epidermis. Studies from male volunteers revealed that skin around facial pores has higher TEWL and more parakeratotic corneocytes. These results suggested that unsaturated fatty acids in excessive sebum might cause the abnormal epidermal differentiation, and this condition might make the pores conspicuous.

### 2. Effects of unsaturated fatty acids on the differentiation of epidermis and the calcium influx into keratinocytes

In order to evaluate the effects of sebum components on the epidermal differentiation, sebum components were applied to the dorsal skin of hairless mice. The application of triglycerides (triolein), saturated fatty acids (palmitic acid and stearic acid) did not induce scaly skin, parakeratosis, and epidermal proliferation. On the other hand, unsaturated fatty acids (palmitoleic acid and oleic acid) induced these abnormal conditions. Oleic acid also disturbed the calcium distribution in the epidermis *in vivo*.

Next, these lipids were applied to normal human keratinocytes cultured *in vitro*. Intracellular calcium concentration [$Ca^{2+}$] was evaluated by a calcium indicator fura-2. Unsaturated fatty acids increased the calcium level, whereas saturated fatty acids and triolein did not.

These results suggest that unsaturated fatty acids in excessive sebum alter the dynamics of calcium concentration in epidermal keratinocytes and thereby induce abnormal differentiation.

### 3. Involvement of NMDA receptors in the function of oleic acid on the epidermal barrier and calcium influx into epidermal keratinocytes

In order to clarify the mechanism of the abnormal epidermal differentiation caused by unsaturated fatty acids, the involvement of calcium channel receptors in the function of oleic acid on the epidermal barrier and calcium influx into cultured keratinocytes was evaluated. First, antagonists of calcium channel receptors were applied to mouse skin. NDMA type glutamate receptor antagonists such as MK801 and D-AP5 specifically inhibited the increase of TEWL caused by oleic acid. These compounds also suppressed epidermal hyper-proliferation.

Next, the effects of these receptor antagonists on calcium influx into cultured normal human keratinocytes were studied. MK801 and D-AP5 suppressed the increase of intracellular [$Ca^{2+}$] induced by oleic acid. MK801 also suppressed the production of inflammatory cytokines such as IL-1α and TNF-α by keratinocytes induced by oleic acid.

These results suggest that the function of oleic acid on epidermal differentiation is associated with the NMDA type glutamate receptors.

### Discussions

Sebaceous glands are located on all parts of human skin except the palms and soles. They present in the greatest number on the face and scalp, whereas sebaceous glands on other parts of the body are far fewer. Barrier function of the facial skin is lower and skin surface texture is less organized in comparison with the rest of the body from the neck below. Although exposure to ultra-violet rays is regarded as the main cause for the impaired condition of facial skin, sebum may be another cause for the impaired function.

Excessive sebum is related to acne vulgaris and conspicuous facial pores. From this study, it was determined that unsaturated fatty acids such as oleic acid in excessive sebum might cause the abnormal epidermal differentiation via the function of NMDA type glutamate receptors. This indicates that by controlling the damage caused by unsaturated fatty acids, the generation of acne vulgaris and conspicuous facial pores can be suppressed. Chemical compounds possessing an ability to control intracellular [$Ca^{2+}$] such as NMDA receptor antagonists can be good candidates for daily application.

On the other hand, the functions of sebum and unsaturated fatty acids are not elucidated enough. An appropriate amount of sebum is thought to have a moisturizing effect on the skin. Indeed, skin with an insufficient amount of sebum tends to be dry. Unsaturated fatty acid has an ability to increase the fluidity of the plasma membrane. Saturated fatty acids have less fluidity and are thought to cause arteriosclerosis.

Unsaturated fatty acids may increase the fluidity of sebum, which helps the secretion from sebaceous glands.

**Publications**

[1] Katsuta Y, Iida T, Inomata S, and Denda M, "Unsaturated fatty acids induce calcium influx into keratinocytes and cause abnormal differentiation of epidermis." *J Invest Dermatol.* 124(5), 1008-1013 (2005)

[2] Katsuta Y, Hasegawa K, Iida T, Inomata S and Denda M, "Function of oleic acid on epidermal barrier and calcium influx into keratinocytes is associated with N-methyl D-aspartate-type glutamate receptors." *Br J Dermatol.* 160(1), 69-74 (2009)